

Example of a Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Con.	Optical Units
Standard 0	(0 µg/ml)	1.69
Standard 1	(0,1 µg/ml)	1.35
Standard 2	(0,5 µg/ml)	0.93
Standard 3	(1 µg/ml)	0.67
Standard 4	(2,5 µg/ml)	0.46
Standard 5	(5 µg/ml)	0.33
Standard 6	(10 µg/ml)	0.23

LIMITATION OF PROCEDURE**1. Interfering Substances**

Any improper handling of samples or modification of this test might influence the results. Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 7.5 mg/ml) have no influence on the assay results.

2. Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of DHEA-S in a sample.

REFERENCES

1. Tietz, N. W., Textbook of Clinical Chemistry, Saunders, 1968

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For Research Use Only. Not for use in Diagnostic Procedures.

**DHEA-S ELISA**

Catalog No. DH079S (96 Tests)

INTENDED TO USE

The Calbiotech DHEA-S Kit is an ELISA for the measurement of DHEA-S in human serum or plasma.

SUMMARY AND EXPLANATION

DHEA-S is a more specific product of the adrenals and measurements of this steroid are widely intended in clinical practice. The clinical importance of plasma assays of DHEA-S is associated with the diagnosis of adrenal hyperplasia and differential diagnosis of hirsutism.

PRINCIPLE OF THE TEST






The DHEA-S ELISA kit is based on the competition principle and the microplate separation. An unknown amount of DHEA-S present in the sample and a fixed amount of DHEA-S conjugated with horse-radish peroxidase compete for the binding sites of a polyclonal DHEA-S antiserum coated onto the wells. After one hour incubation the microtiterplate is washed to stop the competition reaction. Having added the substrate solution the concentration of DHEA-S is inversely proportional to the optical density measured.

MATERIALS PROVIDED	96 Tests
Microwells coated Anti-DHEA-S	12x8x1
Reference Standard set (7 vials ready to use)	1 ml
Enzyme Conjugate(ready to use)	25 ml
TMB Substrate (ready to use)	14 ml
Stop Solution (ready to use)	14 ml
Wash solution 40X	30 ml

MATERIALS NOT PROVIDED

1. A microtiterplate reader (450±10 nm)(e.g. the Instruments Microtiterplate Reader)
2. Precision micropipettes with disposable tips for 20, 100 and 200 µl
3. Standard refrigerator
4. Absorbent paper
5. Deionized water
6. Graph paper

Cat#: DH079S (96 Tests)
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SPECIMEN COLLECTION HANDLING

1. When stored at 2° to 8°C unbroken reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
2. Enzyme-Conjugate, Standard Solution, Substrate Solution, Wash Solution and Zero Standard must be stored at 2° to 8°C.
3. Microtiter wells must be stored at 2° to 8°C. Once the foil bag has been broken, the immuno-reactivity of the coated microtiter wells is stable for approx. 6 weeks in the tightly closed plastic zip pouch containing the desiccant.

WARNINGS AND PRECAUTIONS

CAUTION: Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984).

1. This kit is for Research use Only.
2. Avoid contact with *Stop Solution*, 0.5 M H₂SO₄. It may cause skin irritation and burns.
3. Replace caps on reagents immediately. Do not switch caps.
4. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
5. Do not pipette reagents by mouth. For in vitro diagnostic use only.
6. Do not mix or use components from kits with different lot numbers.

SPECIMEN PREPARATION AND STORAGE

1. Serum or plasma samples may be used in this assay. No special pretreatment of sample is necessary.
2. The specimen may be stored at 2-8° C for up to 24 hours, and should be frozen at -10° C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.
3. Samples suspected to contain DHEA-S concentrations higher than 10 µg/ml are to be diluted with ZeroStandard.

Attention: Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

Wash Solution: Add deionized water to the 40 x concentrated Wash Solution (contents: 30 ml) to a final volume of 1200 ml. The diluted Wash Solution is stable for 2 weeks at room temperature.

ASSAY PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use.

1. Secure the desired number of coated strips in the holder.
2. Dispense 25 µl of each standards, controls and sample with new disposable tips into appropriate wells.
3. Dispense 200 µl of Enzyme-Conjugate into each well.

4. Thoroughly mix the plate for 10 seconds. It is important to have complete mixing in this step.
5. Incubate for 60 minutes at room temperature.
6. Briskly shake out the contents of the wells.
7. Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add 100 µl of Substrate Solution to each well, at timed intervals.
9. Incubate for 15 minutes at room temperature.
10. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well.
11. Determine the absorbance (OD) of each well at 450±10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

Any microwell reader capable of determining the absorbance at 450±10nm may be used.

The DHEA-S value of each serum sample is obtained as follows :

Using linear-linear or semi log graph paper, construct a standard curve by plotting the average absorbance (Y) of each Reference Standard against its corresponding concentration (X) in µg/ml . For construction of the standard curve we recommend a four parameter logistic function. Use the average absorbance of each serum sample to determine the corresponding DHEA-S value by simple interpolation from this standard curve, multiplying by the initial sample dilution, if necessary.

EXPECTED NORMAL VALUES

Normal Women: Premenopausal: 0.8 - 3.9 µg/ml, Term Pregnancy: 0.2 - 1.2 µg/ml, Postmenopausal: 0.1 - 0.6 µg/ml

Normal Men: 1.0 - 4.2 µg/ml

Newborns (both sexes) 1.7 - 3.6 µg/ml

Conversion factor: 1 µg/ml = 2.6 µmol/L